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ORIGINAL INVESTIGATION

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Polymorphisms in DNA double-strand break repair genes and risk of breast cancer: two population-based studies in USA and Poland, and meta-analyses

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Abstract The double-strand break DNA repair pathway has been implicated in breast carcinogenesis. We evaluated the association between 19 polymorphisms in seven genes in this pathway (*XRCC2*, *XRCC3*, *BRCA2*, *ZNF350*, *BRIP1*, *XRCC4*, *LIG4*) and breast cancer risk in two population-based studies in USA (3,368 cases and 2,880 controls) and Poland (1,995 cases and 2,296 controls). These data suggested weak associations with breast cancer risk for *XRCC3 T241M* and *IVS7-14A > G* (pooled odds ratio (95% confidence interval): 1.18 (1.04–1.34) and 0.85 (0.73–0.98) for homozygous variant vs wild-type genotypes, respectively), and for an uncommon variant in *ZNF350 S472P* (1.24 (1.05–1.48)), with no evidence for study heterogeneity. The remaining variants examined had no significant relationships to breast cancer risk. Meta-analyses of studies in Caucasian populations, including ours, provided some support for a weak association for homozygous variants for *XRCC3 T241M* (1.16 (1.04–1.30); total of 10,979 cases and 10,423 controls) and *BRCA2 N372H* (1.13 (1.10–1.28); total of 13,032 cases and 13,314 controls), and no support for *XRCC2 R188H* (1.06 (0.59–1.91); total of 8,394 cases and 8,404 controls). In conclusion, the genetic variants evaluated are unlikely to have a substantial overall association with breast cancer risk; however, weak associations are possible for *XRCC3* (*T241M* and *IVS7-14A > G*), *BRCA2 N372H*, and *ZNF350 S472P*. Evaluation of potential underlying gene–gene interactions or associations in population subgroups will require even larger sample sizes.

Introduction

Double stranded breaks (DSBs) in DNA can be caused by many different factors, including endogenous

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exposure to free radicals produced during normal cellular metabolism, exogenous exposures to chemicals and ionizing radiation (van Gent et al. 2001). DSBs can result in chromosomal aberrations that lead to cell malfunctioning resulting in cell death or tumor genesis (van Gent et al. 2001). Two main repair mechanisms exist to protect cells from these detrimental consequences: DSB homologous recombination (HR) and non-homologous end-joining (NHEJ), which differ in their requirement for a homologous template DNA and in the fidelity of DSB repair (van Gent et al. 2001).

Known breast cancer susceptibility genes (e.g. *BRCA1*, *BRCA2*, *ATM*, *TP53* and *CHK2*) are involved in the repair of DSBs and related processes such as cell cycle control, indicating that inherited deficiencies in this pathway might result in breast cancer predisposition. In addition, epidemiological studies have shown that exposure to ionizing radiation, which is known to cause DSBs, is a risk factor for breast cancer (John and Kelsey 1993). This evidence makes DSB genes good candidates for study in relation to breast cancer susceptibility.

Polymorphisms in DSB genes that could potentially alter function have been evaluated in relation to breast cancer risk in a number of epidemiological studies. The most studied polymorphisms include *XRCC2 R188H* (Han et al. 2004; Kuschel et al. 2002; Rafii et al. 2002; Webb et al. 2005), *XRCC3 T241M* (Figueiredo et al. 2004; Forsti et al. 2004; Han et al. 2004; Jacobsen et al. 2003; Kuschel et al. 2002; Smith et al. 2003a, b; Webb et al. 2005) and *BRCA2 N372H* (Freedman et al. 2004; Healey et al. 2000; Ishitobi et al. 2003; Spurdle et al. 2002). Although several studies have suggested that these polymorphisms are related to modest increases in breast cancer risk, current evidence is still inconclusive and further research is required to clarify the relationships.

In this report, we evaluated whether polymorphisms in genes involved in DNA DSB HR (*XRCC2*, *XRCC3*, *BRCA2*, *ZNF350, BRIP1*) and NHEJ (*XRCC4*, *LIG4*) are associated with breast cancer risk. We analyzed two independent population-based studies of breast cancer in USA and Poland that included a total of 5,363 cases and 5,176 controls, doubling the number of subjects included in previously published studies. In addition, we performed meta-analyses to summarize the current knowledge on the associations between *XRCC2 R188H, XRCC3 T241M, BRCA2 N372H* and breast cancer risk, which included a range of 8,394–13,032 cases in total.

Materials and methods

USA breast cancer study

Eligible cases were all English speaking female residents of Wisconsin, Massachusetts (excluding metropolitan Boston), and New Hampshire, aged 20–74, with a recent primary diagnosis of invasive or in situ (MA and NH only) breast cancer reported to the states' cancer registries during 1998–2001. Control women were randomly selected from population lists (licensed drivers for ages 20–64 years and Medicare beneficiaries for ages 65–74 years) in each state, and frequency matched to the cases for age in 5-year categories. Women provided a telephone interview on demographic characteristics including ethnic ancestry, and on known or suspected breast cancer risk factors. Cases and controls were interviewed concurrently. Following the interview, women were asked if they were willing to provide a buccal cell sample through the mail for collection of DNA. For the first half of the study (June 1998–December 1999), those agreeing were sent a buccal cytobrush kit (two cytobrushes per subject). Because mouthwash samples were later found to provide higher DNA yield and quality than cytobrush samples (Garcia-Closas et al. 2001), participants enrolled in the second half of the study (January 2000–April 2001) were sent a mouthwash collection kit instead of cytobrushes. Samples were returned to a National Cancer Institute (NCI) affiliated laboratory for processing. Collection, storage and DNA isolation protocols have been previously described (Garcia-Closas et al. 2001). The study was reviewed and approved by local institutional review boards (IRB) and the NCI IRB. All participants provided written informed consent.

Approximately 80% of eligible breast cancer cases and 75% of eligible controls agreed to the interview. Participation rates for buccal cell collection among cases and controls who completed the interview, respectively, were 73% and 64% for cytobrush samples, and 71% and 61% for mouthwash samples. Women who did not provide a buccal sample were similar to participants in traditional breast cancer risk factors, although they tended to be older in age (data not shown). Because of insufficient DNA quantity or quality, 595 of 2,097 cases and 588 of 1,993 controls with cytobrush samples and 22 of 1,986 cases and 14 of 1,573 controls with mouthwash samples were excluded from all genotype analyses. To limit heterogeneity of the study population, analyses were further restricted to Caucasian women, most of European ancestry, resulting in a total of 1,470 cases (11% in situ) and 1,366 controls with cytobrush DNA, and 1,898 cases (12% in situ) and 1,514 controls with mouthwash DNA samples.

Polish breast cancer study

A population-based case–control study of breast cancer was conducted in Poland for a 3-year period (January 2000–January 2003). Eligible cases were all women of age 20–74 years, residents of Warsaw and Lodz, and who were newly diagnosed with either histologically or cytologically confirmed in situ or invasive breast cancer. Cases were recruited through a rapid identification system organized at participating hospitals that covered about 90% of all eligible cases. Periodic checks were made against the cancer registries in both cities to assure

complete identification of cases. The Polish Electronic System, a data base with demographic information from all residents of Poland, was used to randomly select controls, stratified by city and age in 5-year categories, on a quarterly basis from January 2000 to September 2003. Women provided a personal interview on known and suspected risk factors. Venous blood samples were collected by a trained nurse. The study protocol was reviewed and approved by local and NCI IRBs. All participants provided written informed consent.

Of the 3,037 eligible cases and 3,639 eligible controls identified, 2,386 (79%) cases and 2,502 (69%) controls agreed to participate in the personal interview. The present study is limited to women with blood DNA samples: 1,995 cases (6% in situ) and 2,296 controls, which represent 84 and 92%, respectively, of the study population. All study participants were of Polish Caucasian origin. Women who did not provide a blood sample tended to be older than participants and were similar in traditional breast cancer risk factors, with the exception of a small but borderline significantly decreased proportion of women who were nulliparous and of current or recent HRT users (data not shown).

Genotyping

Genomic DNA for genotype analyses was isolated from buffy coat or whole blood samples using the Autopure LS® DNA Purification System (Gentra Systems, Inc, Minneapolis, MN, USA). To characterize genetic variation in DSB DNA repair, we selected 19 single nucleotide polymorphisms (SNPs) in seven DSB genes (Table 2). We chose SNPs with an expected rare allele frequency in Caucasians >5% that showed previous evidence of associations with breast cancer risk or had possible functional significance, such as mis-sense variants, with assays available at the time of analysis at the Core Genotyping Facility (CGF) of the Division of Cancer Epidemiology and Genetics, NCI. Other SNPs were chosen to provide additional variants within a gene, or to capture haplotype diversity. For *ZNF350*, SNPs were chosen to tag common haplotypes, namely those with greater than 2% frequency (Rutter et al. 2003), based on SNPHAP analysis as implemented in Haploview (Zhang et al. 2002). Duplicated DNA samples (150 cytobrush and 187 mouthwash DNA pairs in USA and 100 DNA pairs in Poland) showed ≥99% concordance for all but four assays: *ZNF350 R568S* (98%) and *BRIP1 S919P* (95%) in the USA study, and *BRCA2 N372H* (98%), *XRCC3 Ex2+2A>G* (96%) and *IVS7-14A>G* (90%) in the Polish study. We observed no significant departures from Hardy–Weinberg equilibrium in the control populations for all but one assay in the USA study: *LIG4 T9I* had a small excess of homozygous variants (42 (3.9%) observed versus 31.2 (2.9%) expected, $P=0.02$). Description and methods for each genotype assay can be found at http://www.snp500cancer.nci.nih.gov (Packer et al. 2004).

Statistical analyses

Odds ratios (OR) and 95% CI from logistic regression models with dummy variables for matching factors (age in 5-year categories and study site (WI, MA, NH, Lodz or Warsaw)) were used to estimate relative risks for the genotypes examined. Age and site-adjusted estimates were very similar to crude estimates. Further adjustment for education, age at menarche, parity, type of menopause, age at first full-term pregnancy, age at menopause, body mass index, family history of breast cancer, and personal history of benign breast disease did not appreciably alter relative risk estimates. Therefore, only age and site-adjusted estimates are presented. The association between genotypes and breast cancer risk was tested using a 2 degrees of freedom (df) likelihood ratio test (LRT), and a trend test. Heterogeneity of genotype ORs among groups of women defined by age and family history of breast cancer were evaluated by introducing interaction terms in logistic regression models.

Associations between genotypes and breast cancer risk were initially evaluated among women participating in the USA study who provided a mouthwash sample. In order to reduce cost, only SNPs with a suggestion of an association in the USA study (P for trend or LRT (2df) < 0.20), or with significant ($P<0.05$) associations in previously published studies, were evaluated in the Polish study. Because of the limited amount of DNA obtained from cytobrushes in the USA study, these samples were only genotyped for assays significantly ($P<0.05$) associated with risk in women with a mouthwash sample or in the Polish study. Differences in genotype odds ratios estimated from the three different study population groups (USA mouthwash, USA cytobrush and Polish) were tested with a LRT with 2df when using data from the USA mouthwash and Polish studies, or 3df when using data from all three population subgroups.

Haplotype frequencies, ORs and 95%CIs were estimated using HaploStats (http://www.mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm). Haplotype ambiguity due to unknown linkage phase of the analyzed genotypes was accounted for by modeling the probabilities of the possible haplotype pairs per subject (Schaid 2004).

Meta-analyses

Epidemiological studies investigating polymorphisms in DSB DNA repair and breast cancer risk, published through August 2005, were identified in a Medline search and used for meta-analyses. A random-effects model was used to obtain summary OR (95% CI) calculated by weighting each study result by a factor of within- and between-study variance (Laird and Mosteller 1990). Summary measures were based on crude OR (95% CI) from individual studies calculated

from published frequency tables (i.e. *XRCC2 R188H* and *XRCC3 T241M*), or on reported OR (95% CI) when frequencies were not presented in all publications (i.e. *BRCA2 N372H*). The presence of overall heterogeneity across studies was assessed by the *Q* test (Laird and Mosteller 1990). We also explored differences by subgroups of studies defined by geographical region and ethnicity. Publication bias was assessed by Begg (Begg and Mazumdar 1994) and Egger's tests (Egger et al. 1997). Statistical analyses were done with STATA (Version 8.2, Special Edition).

Results

Demographic and reproductive characteristics of the two control populations in USA and Poland are shown in Table 1. Compared to women in the Polish study, women in the USA study were more likely to have a higher level of education, earlier age at menarche, higher number of full-term births, earlier age at first full-term birth, a history of oral contraceptive use, surgical menopause, earlier age at menopause, a history of hormone replacement therapy use, lower BMI, a family history of breast cancer, and a personal history of benign breast disease. Breast cancer cases were diagnosed at an earlier age in the USA than in the Polish study (mean ± SD 54 ± 9 and 56 ± 10, respectively, $P < 0.001$). Both study populations demonstrated associations with most established life-style and reproductive or hormonal risk factors for breast cancer that were similar in direction and magnitude to those reported in other populations (data not shown).

We analyzed 19 SNPs in 7 DSB DNA repair genes (Table 2). These were initially evaluated in women with mouthwash DNA in the USA study, and 8 SNPs with no evidence of an association with breast cancer risk were not further evaluated (Table 3). Homozygotes for the uncommon variant in *XRCC2 R188H* showed a reduced risk for breast cancer, particularly in the Polish population. However, the association in the pooled data was only borderline significant. A suggestion of an association for the *XRCC3 Ex2+2A>G* (5′ UTR) polymorphism and breast cancer risk in women from the USA study with mouthwash DNA, was not confirmed in the Polish study (LRT (2df) for study heterogeneity $P = 0.09$; Table 4). Subjects who were heterozygous or homozygous for the variants for *XRCC3 IVS7-14A>G* showed an inverse association with breast cancer risk in the Polish population with a significant trend, which was also present in the pooled analysis of Polish and USA studies (LRT (2df) for study heterogeneity $P = 0.49$; Table 4). Women with the homozygous variant *XRCC3 T241M* genotype did not have a significant increase in risk compared to women with the wild-type homozygous genotype in the USA study (mouthwash and cytobrush samples combined); however, a small but significant increase in risk was observed for women in the Polish study (Table 4). When all data were combined, we observed a small increase in risk for *T241M* homozygous variants, with no evidence for study heterogeneity (LRT (3df) $P = 0.67$; Table 4).

In both USA and Polish populations, we observed four common and four rarer haplotypes inferred from the three *XRCC3* SNPs (*Ex2+2A>G*, *IVS-14A>G*, *T241M*) analyzed. The global test for haplotype effects was significant in both study populations ($P = 0.02$ for both populations combined). Subjects with the AGC haplotype (31% cases and 34% controls) had an inverse association with breast cancer risk compared to subjects with the most common haplotype (AAT, 38% cases and 36% controls), with OR (95% CI) = 0.90 (0.84–0.96). The estimated frequency for a rare haplotype (GAT) was higher for cases (0.12%) than controls (0.06%); however, reliable estimates of risk could not be obtained because of the rarity of this haplotype in USA and Polish populations.

The *BRCA2 N372H* variant was not significantly associated with breast cancer risk in the USA study (mouthwash and cytobrush data combined), but a weak association with a significant trend was found in the Polish data (Table 4). When data were combined, we observed no significant association between this polymorphism and breast cancer risk, with no evidence for study heterogeneity (LRT (3df) $P = 0.23$; Table 4). Women heterozygous for *BRCA2 S2414S* had a small but significant increase in risk compared to homozygous wild-type women with mouthwash samples in the USA study that was not confirmed in the Polish study (LRT (3df) $P = 0.09$; Table 4).

Data from women with mouthwash DNA in the USA study suggested an association between two SNPs in *ZNF350* (*D35D* and *S472P*) and breast cancer risk (data not shown). These two SNPs were then analyzed in women with cytobrush DNA and in the Polish study. Genotype analyses using combined data from women with mouthwash and cytobrush DNA, showed a significantly ($P = 0.005$) increased breast cancer risk for *ZNF350 S472P* heterozygotes compared to the homozygous wild-type genotype (only two cases and five controls carried the homozygous variant genotype). This genotype was not significantly related to risk in the Polish study; however, differences between studies were not statistically significant (LRT (3df) = 0.24; Table 4), and pooling data from both studies suggested a weak association (1.2 (1.1–1.5), $P = 0.01$). Four (*D35D*, *L66P*, *P373P*, *S472P*) of the five *ZNF350* SNPs analyzed formed a haplotype block of linkage disequilibrium (LD) as defined by the solid spline of LD and four gamete rule in Haploview (Zhang et al. 2002). Pairwise D' values between these four SNPs were > 0.99 in the USA and Polish studies. Haplotype analyses based on these four *ZNF350* SNPs showed associations between two rare haplotypes (CCCT and CTCC) compared to the common haplotype (TTCT) in the USA study, but not in the Polish study (Table 5). The association between the CCCT haplotype and breast cancer risk was stronger for early (less than 50 years of age) than later

Table 1 Characteristics of the populations in the USA and Polish breast cancer studies, based on 2,880 USA and 2,296 Polish control women

Study characteristic	USA Study		Polish Study		*P*
	N	%	*N*	%	
Education level					
Less than high school	191	7	878	38	
High school	1,113	39	879	38	
Some tech training or college	767	27	188	8	
College degree	809	28	342	15	< 0.001
Age at menarche					
< 12	1,235	43	514	23	
13	848	30	530	23	
14	430	15	637	28	
15	184	6	258	11	
≥ 16	163	6	330	15	< 0.001
Number of full-term births					
Nulliparous	331	12	253	11	
1	302	10	682	30	
2	878	31	1,016	44	
≥ 3	1,366	47	345	15	< 0.001
Age at first full-term birth among parous women					
< 20	483	19	286	14	
20–24	1,226	48	1,050	51	
25–29	640	25	511	25	
≥ 30	192	8	196	10	< 0.001
Oral contraceptive use					
Never	1,285	45	2,019	89	
Ever	1,590	55	255	11	
Menopausal status					
Pre-menopausal	982	34	656	29	
Post-menopausal	1,701	59	1,541	67	
Unclear	197	7	97	4	< 0.001
Age at menopause among post-menopausal women					
< 45	571	37	213	14	
45–49	499	32	477	31	
≥ 50	494	32	846	55	< 0.001
Hormone replacement therapy use among post-menopausal women					
Never	778	46	1,193	82	
Former	776	46	99	7	
Current	146	9	169	12	0.01
Current BMI among pre-menopausal women					
< 25	540	56	300	46	
25 to < 30	258	27	217	33	
≥ 30	173	18	139	21	< 0.001
Current BMI among post-menopausal women					
< 25	744	44	403	26	
25 to < 30	589	35	574	37	
≥ 30	359	21	5,764	37	< 0.001
Family history of breast cancer in first-degree relatives					
No	2,451	86	2,163	94	
Yes	384	14	133	6	0.04
History of benign breast disease					
No	2,150	75	2,109	93	
Yes	718	25	165	7	< 0.001

Differences in numbers in table and total numbers of controls in each study are due to missing information

(≥50 years of age) age at breast cancer diagnosis (P interaction = 0.04) in the US, but not in the Polish study (data not shown). None of the other associations evaluated were significantly modified by age or family history (data not shown).

Meta-analyses

We performed a meta-analysis of the association between *XRCC2 R188H* and breast cancer risk that included data from the USA and Polish studies in this

Table 2 Genes and single nucleotide polymorphisms (SNP) in double strand break DNA repair evaluated in the USA and Polish breast cancer studies

Gene	Function in DSB repair	Chromosomal location	Nucleotide change	Amino acid change	SNP500 ID[a]	dbSNP ID	Minor allele frequency in control population	
							US	Poland
Homologous recombination								
XRCC2	X-ray repair complementing defective repair in Chinese hamster cells 2 - member of the RecA/Rad51-related protein family that participates in homologous recombination to maintain chromosome stability and repair DNA damage	7q36.1	Ex3 + 442G > A	R188H	XRCC2-01	rs3218536	0.08	0.07
XRCC3	X-ray repair complementing defective repair in Chinese hamster cells 3 - member of the RecA/Rad51-related protein family that participates in homologous recombination to maintain chromosome stability and repair DNA damage	14q32.3	Ex2 + 2A > G		XRCC3-04	rs1799794	0.18	0.21
			IVS7-14A > G		XRCC3-03	rs1799796	0.31	0.37
			Ex8-53C > T	T241M	XRCC3-01	rs861539	0.38	0.34
BRCA2	Involved in the orchestration of the Rad51 response	13q12.3	Ex10 + 72A > C	N289H	BRCA2-10	rs766173	0.04	NA
			Ex10 + 321A > C	N372H	BRCA2-01	rs144848	0.28	0.26
			Ex11 + 1487A > G	K1132K	BRCA2-02	rs1801406	0.30	0.34
			Ex14-194A > G	S2414S	BRCA2-04	rs1799955	0.20	0.23
			Ex27-336A > C		BRCA2-06	rs15869	0.21	NA
ZNF350	Originally termed ZBRK1–BRCA1-interacting protein	19q13.41	Ex3-38T > C	D35D	ZNF350-01	rs4986773	0.26	0.25
			Ex4-42T > C	L66P	ZNF350-02	rs2278420	0.26	0.18
			Ex5 + 881C > A	P373P	ZNF350-07	rs3764538	0.13	0.16
			Ex5-699T > C	S472P	ZNF350-03	rs4986771	0.03	0.02
			Ex5-610A > T	R568S	ZNF350-04	rs2278415	0.14	0.15
BRIP1	Originally termed BACH1–BRCA1-interacting protein	17q22-q24	Ex19-151T > C	S919P	BRIP1-02	rs4986764	0.43	NA
			IVS12-47C > G		BRIP1-10	rs4988351	0.24	NA
Non-homologous end-joining								
XRCC4	Forms a complex with LIG4 involved in the ligation of DNA ends	5q13-q14	IVS7-1A > G		XRCC4-01	rs1805377	0.11	NA
LIG4	Forms a complex with XRCC4 involved in the ligation of DNA ends	13q33-q34	Ex2 + 54C > T	T9I	LIG4-02	rs1805388	0.17	NA
			Ex2-1349T > C	D568D	LIG4-01	rs1805386	0.18	NA

NA not applicable

[a]See http://www.snp500cancer.nci.nih.gov for assay information

Table 3 Polymorphisms in genes involved in double strand DNA break repair and breast cancer risk. Based on data from 1,898 cases and 1,514 controls with mouthwash DNA in the USA breast cancer study

Gene	SNP	Genotype	Cases	Controls	OR[a]	95%	CI	LRT (2df)
BRCA2	*N289H*	*Asn/Asn*	1,460	1,145	1.00			
		Asn/His	112	94	0.93	0.70	1.23	
		His/His	3	3	0.78	0.16	3.88	0.83
		P for trend			0.55			
	K1132K	*AA*	821	679	1.00			
		AG	763	575	1.11	0.95	1.29	
		GG	160	127	1.04	0.81	1.34	0.40
		P for trend			0.34			
	Ex27-336A > C	*AA*	1,084	868	1.00			
		AC	565	468	0.98	0.84	1.14	
		CC	92	60	1.23	0.88	1.73	0.43
		P for trend			0.60			
BRIP1	*S919P*	*Pro/Pro*	529	406	1.00			
		Pro/Ser	761	612	0.96	0.81	1.13	
		Ser/Ser	306	236	0.98	0.79	1.22	0.88
		P for trend			0.81			
	IVS12 −47C > G	*CC*	754	607	1.00			
		CG	486	389	0.99	0.84	1.18	
		GG	87	60	1.17	0.82	1.65	0.67
		P for trend			0.61			
XRCC4	*IVS7-1A > G*	*AA*	1,231	964	1.00			
		AG	285	239	0.95	0.78	1.15	
		GG	20	10	1.56	0.72	3.34	0.42
		P for trend			0.99			
LIG4	*T9I*	*Thr/Thr*	920	724	1.00			
		Thr/His	339	277	0.96	0.79	1.15	
		His/His	57	42	1.06	0.70	1.61	0.85
		P for trend			0.88			
	D568D	*CC*	904	714	1.00			
		CT	379	309	0.98	0.82	1.17	
		TT	55	34	1.28	0.82	1.99	0.51
		P for trend			0.63			

Differences between the total numbers of cases and controls and frequencies shown in the table are due to missing genotype data

[a]Adjusted for age and region

report and four previously published studies among Caucasian women in the UK, Australia and USA (Han et al. 2004; Kuschel et al. 2002; Rafii et al. 2002; Webb et al. 2005) (total of 8,394 cases and 8,404 controls). The summary estimates showed no evidence of a substantial association, (OR (95% CI) of 0.97 (0.88–1.07) $P = 0.53$ and 1.06 (0.59–1.91) $P = 0.84$ for *Arg/His* and *His/His*, respectively, compared to *Arg/Arg*), although a small association for the *His/His* genotype could not be ruled out. There was no significant study heterogeneity (Q test $P = 0.33$ and 0.07 for *Arg/His* and *His/His*, respectively), and no evidence of publication bias according to the Egger's or Begg's tests (data not shown).

A meta-analysis on *XRCC3 T241M* and breast cancer risk using data from the USA and Polish studies in this report, and eight previously published studies in Caucasian women (Figueiredo et al. 2004; Forsti et al. 2004; Han et al. 2004; Jacobsen et al. 2003; Kuschel et al. 2002; Smith et al. 2003a, b; Webb et al. 2005) (total of 10,979 cases and 10,423 controls), suggested a small increase in risk for the *Met/Met* compared to the *Thr/Thr* genotype (OR (95% CI) of 1.16(1.04–1.30) $P = 0.009$; Fig. 1). There was no statistically significant study heterogeneity (Q test $P = 0.15$), and no evidence of publication biases according to the Egger's or Begg's tests (data not shown). Although the summary OR was higher for European (4,937 cases and 4,992 controls) than USA (4,344 cases and 4,369 controls) populations (Fig. 1), differences were not statistically significant ($P = 0.25$).

A meta-analysis of *BRCA2 N372H* and breast cancer risk was based on data from the US and Polish studies in this report, and eight previously published studies in predominantly Caucasian populations (Cox et al. 2005; Freedman et al. 2004; Healey et al. 2000; Spurdle et al. 2002) (total of 11,317 cases and 10,812 controls). The summary OR suggested that homozygous variant genotypes for *BRCA2 N372H* have a small increase in breast cancer risk (1.16(1.02–1.33) $P = 0.03$; Fig. 2). Inclusion of data from a multiethnic study in USA (25% Caucasian cases) (Freedman et al. 2004) with 1,715 cases and 2,502 controls resulted in an even weaker summary estimate (1.13(1.01–1.28), $P = 0.04$; Q test $P = 0.21$; Fig. 2).

Although the Q test for overall study heterogeneity in the association between *BRCA2 N372H* homozygous variants and breast cancer was not significant ($P = 0.21$), we observed significant differences when analyses were confined to data from studies in Europe or in USA ($P = 0.01$). While the three studies in USA populations (total of 5,908 cases and 7,587 controls) provided no evidence (0.97(0.85–1.12) $P = 0.68$), Q test $P = 0.77$), six studies in European Caucasian populations suggested a small increase in risk for homozygous variants (total of 5,755 cases and 5,009 controls: (1.27(1.09–1.48)

Table 4 Polymorphisms in four genes involved in double strand DNA break repair and breast cancer risk in the USA (3,368 cases and 2,880 controls) and Polish (1,995 cases and 2,296 controls) studies

Gene	SNP	Genotype	USA study					Polish Study					Study heterogeneity LRT[b]	USA and Polish pooled analysis			
			Cases	Controls	OR	95%	CI	Cases	Controls	OR	95%	CI		OR	95%	CI	LRT
XRCC2	*R188H*	*Arg/Arg*	1,496	1,177	1.00			1,763	1,983	1.00				1.00			
		Arg/His	264	214	0.98	0.80	1.19	212	281	0.85	0.70	1.02		0.90	0.79	1.04	
		His/His	10	11	0.75	0.31	1.79	6	16	0.42	0.16	1.07	0.42	0.57	0.30	1.06	0.07
		P for trend			0.65					0.02				0.04			
XRCC3	*Ex2+2A>G*[a]	*AA*	980	837	1.00			1210	1,386	1.00				1.00			
		AG	521	357	1.24	1.05	1.46	632	736	0.98	0.86	1.12		1.08	0.97	1.19	
		GG	63	52	1.03	0.71	1.51	78	96	0.93	0.68	1.26	0.09	0.97	0.76	1.23	0.34
		P for trend			0.05					0.63				0.26			
	IVS7-14A>G[a]	*AA*	775	602	1.00			882	920	1.00				1.00			
		AG	648	525	0.96	0.82	1.12	847	1,028	0.86	0.75	0.98		0.90	0.81	0.99	
		GG	159	133	0.93	0.72	1.20	254	332	0.80	0.66	0.96	0.49	0.85	0.73	0.98	0.03
		P for trend			0.51					0.006				0.01			
	T241M	*Thr/Thr*	1,102	973	1.00			785	980	1.00				1.00			
		Thr/Met	1,419	1,213	1.03	0.92	1.16	907	1,039	1.09	0.96	1.24		1.06	0.97	1.15	
		Met/Met	457	368	1.09	0.92	1.28	282	266	1.33	1.09	1.61	0.67	1.18	1.04	1.34	0.03
		P for trend			0.33					0.006				0.06			
BRCA2	*N372H*	*Asn/Asn*	1,617	1,412	1.00			1,007	1,239	1.00				1.00			
		Asn/His	1,278	1,057	1.06	0.95	1.18	826	897	1.13	1.00	1.28		1.09	1.01	1.19	
		His/His	266	232	1.00	0.82	1.20	135	140	1.20	0.93	1.54	0.23	1.06	0.91	1.24	0.10
		P for trend			0.57					0.03				0.08			
	S2414S[a]	*AA*	1,046	898	1.00			1,169	1,358	1.00				1.00			
		AG	619	440	1.22	1.05	1.42	700	810	1.01	0.88	1.14		1.09	0.99	1.20	
		GG	78	64	1.05	0.74	1.48	114	108	1.24	0.94	1.63	0.09	1.17	0.94	1.45	0.13
		P for trend			0.05					0.32				0.04			
ZNF350	*D35D*	*TT*	1,554	1,396	1.00			1,083	1,282	1.00				1.00			
		TC	1,159	931	1.12	1.00	1.26	754	835	1.1	0.9	1.2		1.10	1.01	1.20	
		CC	211	186	1.01	0.81	1.24	133	156	1.0	0.8	1.3	0.45	1.01	0.86	1.18	0.09
		P for trend			0.21					0.45				0.08			
	L66P	*Leu/Leu*	2,218	1,914	1.00			1,332	1,540	1.00				1.00			
		Leu/Pro	881	702	1.08	0.96	1.22	580	660	1.02	0.89	1.17		1.06	0.97	1.15	
		Pro/Pro	82	82	0.85	0.62	1.17	66	83	0.91	0.65	1.27	0.84	0.88	0.70	1.11	0.23
		P for trend			0.60					0.92				0.67			
	P373P	*CC*	2,368	2,019	1.00			1,421	1,636	1.00				1.00			
		CA	716	617	0.99	0.88	1.12	517	585	1.02	0.89	1.17		1.00	0.91	1.10	
		AA	55	49	0.93	0.63	1.38	44	60	0.83	0.56	1.24	0.43	0.88	0.67	1.17	0.68
		P for trend			0.77					0.82				0.99			
	S472P	*Ser/Ser*	2,719	2,382				1,908	2,197	1.00				1.00			
		Ser/Pro	241	155	1.35	1.09	1.66	76	87	1.02	0.74	1.39	0.24	1.24	1.05	1.48	0.01
		Pro/Pro	2	5	–					–							
	R568S	*Arg/Arg*	2,318	1,966	1.00			1,413	1,630	1.00				1.00			
		Arg/Ser	742	640	0.98	0.87	1.11	518	586	1.02	0.89	1.18		1.00	0.91	1.09	
		Ser/Ser	59	45	1.12	0.76	1.66	45	61	0.84	0.57	1.25	0.48	0.97	0.74	1.28	0.97
		P for trend			0.98					0.84				0.77			

Odds ratios (OR) and 95% CIs are adjusted for age and region

[a]Genotyped only in mouthwash DNA samples from the USA breast cancer study (1,898 cases and 1,514 controls)

[b]Likelihood ratio test for differences in genotype odds ratios from the three different study population groups (USA mouthwash, USA cytobrush and Polish)

Table 5 Analysis of inferred haplotypes in *ZNF350* based on four htSNPs in the USA (3,368 cases and 2,880 controls with mouthwash or cytobrush DNA) and Polish breast cancer (1,995 cases and 2,296 controls) studies

ZNF350[a]				USA study						Polish study					
				Haplotype frequency		OR	95% CI		*P*	Haplotype frequency		OR	95% CI		*P*
				Cases	Controls					Cases	Controls				
T	T	C	T	0.70	0.71	1.0				0.71	0.72	1.0			
C	T	C	T	0.08	0.08	1.0	0.9	1.2	0.89	0.07	0.06	1.2	1.0	1.4	0.10
C	C	C	T	0.04	0.03	1.3	1.0	1.6	0.04	0.03	0.03	1.0	0.8	1.4	0.86
C	C	A	T	0.14	0.15	1.0	0.9	1.1	0.94	0.17	0.17	1.0	0.9	1.1	0.97
C	T	C	C	0.04	0.03	1.3	1.0	1.5	0.02	0.02	0.02	1.0	0.8	1.4	0.87
					Global $P = 0.06$						Global $P = 0.49$				

[a]Polymorphic bases in 5′ to 3′ order (*D35D, L66P, P373P, S472P*)

$P = 0.002$, Q-statistic $P = 0.67$). We found no evidence of publication bias according to Begg's or Egger's tests, overall or within population subgroups (data not shown).

Discussion

We evaluated associations between common variants in seven genes involved in DSB DNA repair and breast cancer risk, in two large population-based case-control studies conducted in USA and Poland (5,363 cases and 5,176 controls in total). Analyses indicated that the evaluated polymorphisms are unlikely to have a substantial association with overall breast cancer risk. Meta-analyses including this and other published data in Caucasian populations indicated that previously suggested associations for *XRCC3 T241M*, *BRCA2 N372H, XRCC2 R188H* homozygous variants and breast cancer risk are very weak or not present.

A modest association between the homozygous variant genotype for *XRCC3 T241M* and breast cancer risk was first reported in a study in the UK (Kuschel et al. 2002); however, subsequent studies in Caucasian populations were unable to confirm this association (Figueiredo et al. 2004; Forsti et al. 2004; Han et al. 2004; Jacobsen et al. 2003; Smith et al. 2003a, b; Webb et al. 2005). Analyses of combined data from the current USA and Polish studies and a meta-analysis including this and previously published data were consistent with a very small increase in risk for *T241M* homozygous variants, with no evidence for study heterogeneity. This amino acid is evolutionarily conserved and functional data supports that it could be a risk allele for breast cancer (Au et al. 2003; Savas et al. 2004). However, further evidence from both epidemiological and functional studies is required to confirm or rule out a weak association with overall breast cancer risk.

Results from analyses of other *XRCC3* SNPs have also been apparently inconsistent. Although homozygous variants for *XRCC3 IVS7-14A > G* were associated with a significant decrease in breast cancer risk in a UK population (Kuschel et al. 2002) and the current Polish study, this was not confirmed in a previous study conducted in USA (Han et al. 2004) nor in the current USA study (mouthwash DNA only). The *XRCC3 Ex2 + 2A > G* (5′ UTR) polymorphism was not significantly associated with breast cancer risk in the combined analysis of USA and Polish data, nor in two previously published studies (Han et al. 2004; Kuschel et al. 2002). Previous analyses of haplotypes inferred from *Ex2 + 2A > G* (5′ UTR), *T241M*, and *IVS7-14A > G* polymorphisms in *XRCC3* suggested a substantial increase in breast cancer risk associated with the uncommon (0.3%) GAT haplotype (Kuschel et al. 2002). This haplotype was also more common for cases (0.12%) than controls (0.06%) for the combined data from the USA and Polish studies; however, because of

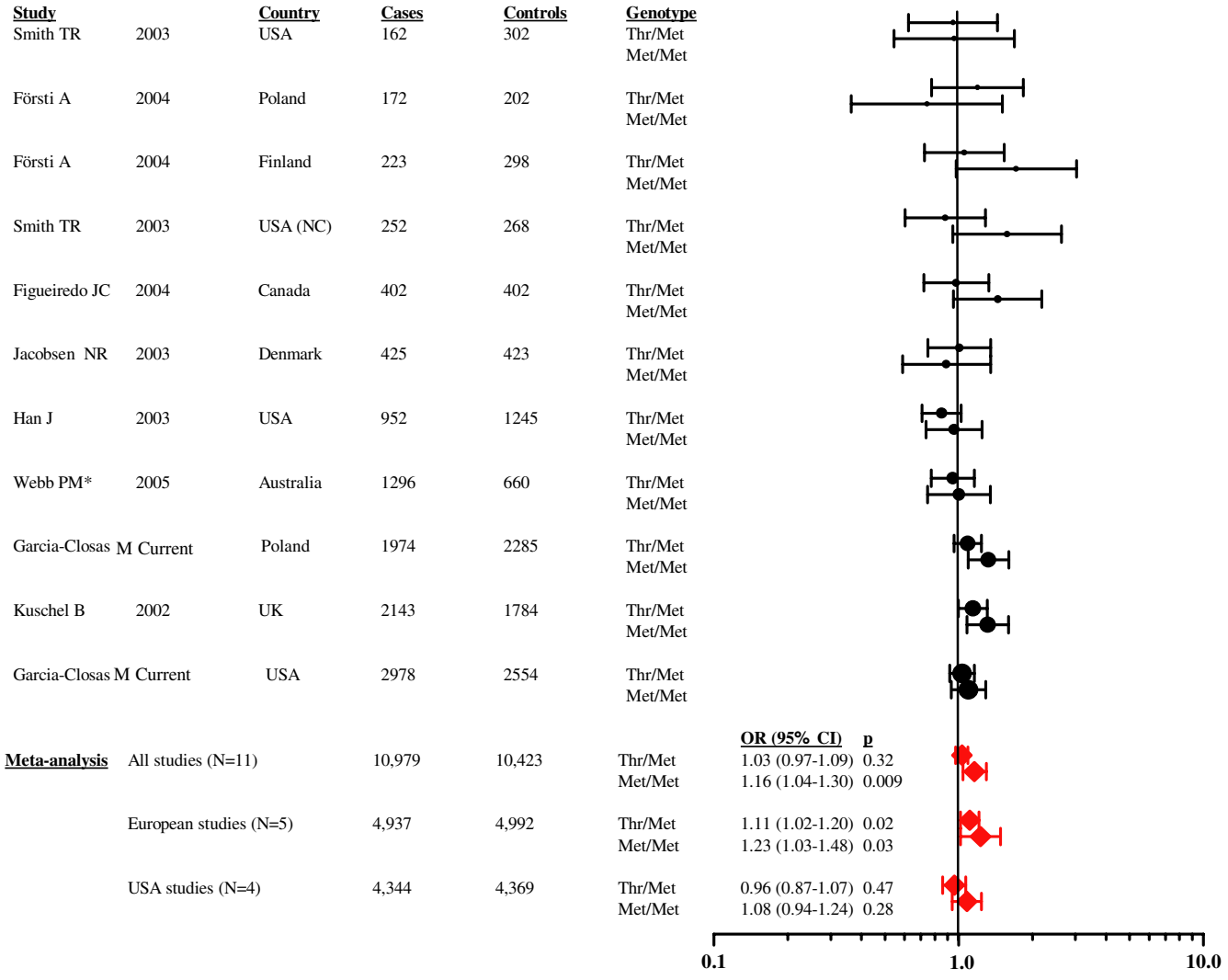

Fig. 1 Meta-analysis of studies on *XRCC3 T241M* and breast cancer risk that included predominantly white populations. *Thr/Thr* is the reference category. Numbers of cases and controls are subjects with genotype information. Webb et al. (2005) estimates were recalculated from data on white women only

the very low estimated frequency, precise estimates of risk could not be obtained.

Homozygous carriers of the *BRCA2 N372H* polymorphism were first reported to have a 30% increase in breast cancer risk in a pooled analysis from five case-control studies conducted among Northern European Caucasian populations (3,459 cases in total) (Healey et al. 2000). This finding was later confirmed in an Australian population (1,397 cases) (Spurdle et al. 2002), but not in two reports from USA (one with 1,715 cases of different ethnicities (Freedman et al. 2004) one with 1,313 Caucasian cases (Cox et al. 2005)), nor in the combined analyses from the current USA and Polish studies. Summary estimates from a meta-analysis of studies in Caucasian populations, suggested the presence of a weak association, which was evident only for studies conducted in Europe, but not for studies conducted in USA (*P* for heterogeneity between USA and European studies = 0.01). True geographical differences in genotype relative risks are unlikely, unless there is a strong unidentified effect modifier associated with geographical location. Because of the weakness of the association, the unexplained evidence for heterogeneity by geographical location, and the unknown functional significance of this polymorphism, further research is needed to confirm or rule out this association.

Previous suggestions for an increased breast cancer risk associated with the rare variant in *XRCC2 R188H* (Kuschel et al. 2002; Rafii et al. 2002), were not confirmed in our two population-based studies nor in two previous studies (Han et al. 2004; Webb et al. 2005). Pooled and meta-analyses presented here did also not provide significant evidence of an association.

Genetic variation in two genes coding for *BRCA1* interacting proteins, *ZNF350* and *BRIP1*, has been recently described (Rutter et al. 2003). An uncommon

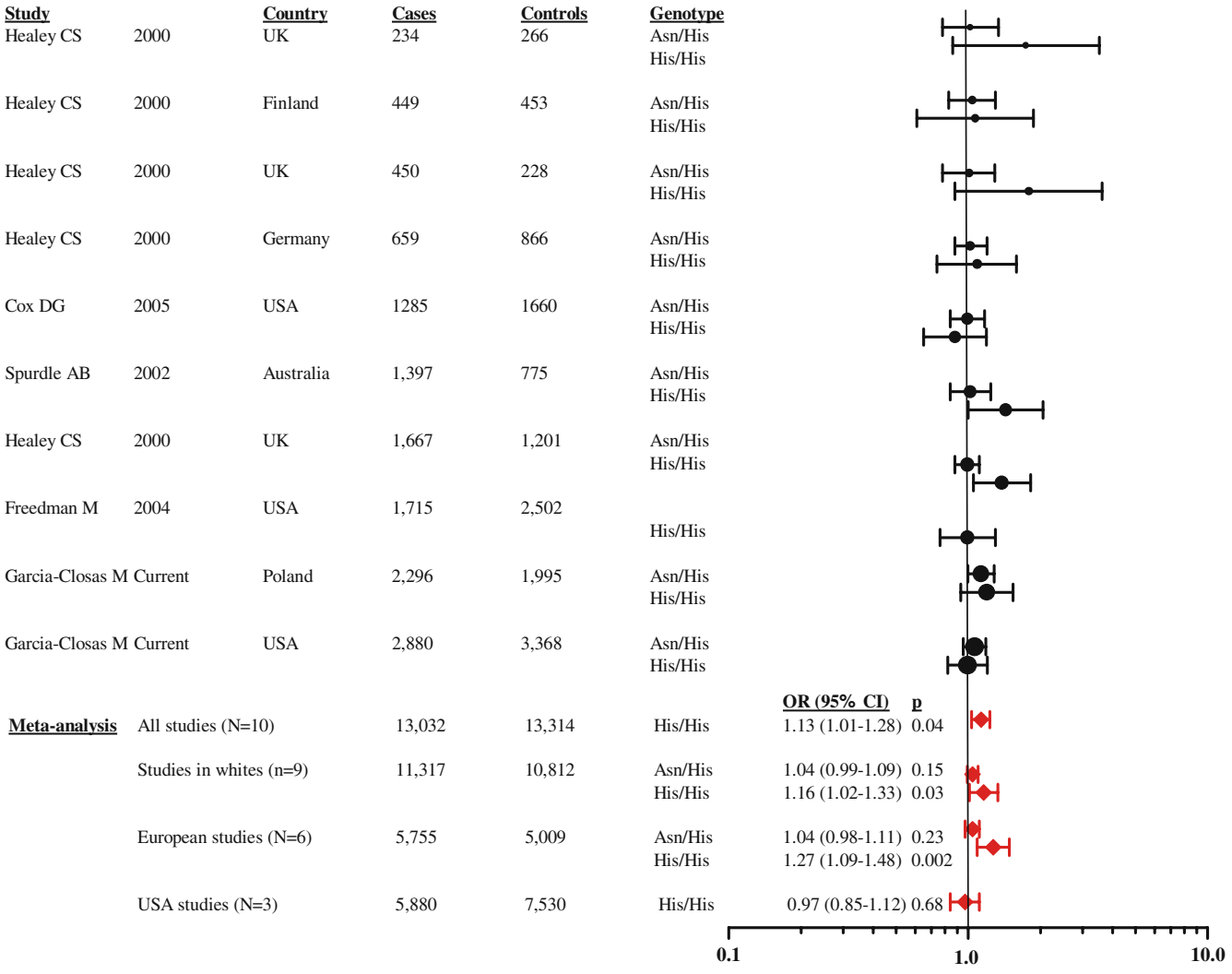

Fig. 2 Meta-analysis of studies on *BRCA2 N372H* and breast cancer risk. *Asn*/*Asn* is the reference category. Numbers of cases and controls are subjects with genotype information. Freedman et al. (2004) is the only study population that is not predominantly white (multiethnic population with approximately 25% whites) and that only presented estimates of association for *His*/*His* vs *Asn*/*Asn*

mis-sense variant in *ZNF350* (*S472P*) was associated with a significant small increase in breast cancer risk in an analysis of pooled data from the USA and Polish studies. This polymorphism has not been previously evaluated in relation to breast cancer risk, except for a kin-cohort study with 190 breast cancer cases where no significant association was observed (Sigurdson et al. 2004). *BRIP1 P919S* was found to increase familial breast cancer risk in the kin-cohort study (Sigurdson et al. 2004), but not in our USA breast cancer study.

We found no significant associations between SNPs in the two NHEJ DSB genes evaluated. The *LIG4 D568D* polymorphism was found to be related to decreased breast cancer risk in a UK population (Kuschel et al. 2002), but not in a USA study (Han et al. 2004) nor our study population in USA. The *LIG4 T9I* and *XRCC4 IVS7-1A > G* were not related to risk in our study in USA nor in a previous Taiwanese study (Fu et al. 2003).

Both the USA and Polish studies have among the highest participation rates attained in population-based studies with collection of biological specimens (Morton et al. 2005). However, collection of DNA samples inevitably reduces overall participation rates and could introduce selection bias. This bias is unlikely to be of relevance in this report because the observed allele frequencies were similar across study populations and to previously published studies, and known breast cancer risk factors were identified in both study populations (data not shown). The study populations were of homogenous ethnic background, particularly the Polish population, thus reducing the possibility of bias due to population stratification. The availability of two large and independent study populations also facilitated the evaluation of the validity of findings, since potential biases that could explain small associations are unlikely to be the same in both populations. Indeed, even though the two study populations were quite different with

respect to demographic and other risk factor characteristics, we observed no significant differences in genotype relative risk estimates, which lend further credibility to the findings.

To reduce cost and DNA usage, the mouthwash DNA samples from the USA study were used to screen for potentially interesting associations that were then followed up in the Polish population and the USA population with very limited quantities of cytobrush DNA. The screening population provided adequate power to detect small associations for the range of allele frequencies in genes evaluated in this report. For instance, it provided 88% power to detect an odds ratio of 1.3 for a genotype with 5% allele frequency under a log-additive model (α-level = 0.2). The power to detect recessive associations was more limited, and thus it is possible that we missed small to moderate recessive associations for some of the more uncommon genotypes, as reflected by the confidence intervals (CIs) shown in Table 3. To further reduce the chance of false negative findings, genotypes that showed no evidence of an association in the screen population but that were previously found to be related to breast cancer, were also analyzed in the Polish population. For genotypes analyzed in the whole study population (USA and Polish combined), we had adequate power to detect very small associations, e.g. 85% power to detect a 1.2 OR with α-level = 0.05. Therefore, it is unlikely that we missed important associations in our analyses. However, in spite of the relatively large sample sizes of our studies, the power to evaluate gene–gene and gene–environment interactions was limited because of the very small magnitudes of the overall associations, and the relatively low frequency of putative susceptibility/protective genotypes (ranging from 0.8% for *XRCC2 R188H* to 14% for *XRCC3 T241M*). A limitation of our investigation is that, except for *ZNF350*, we did not include a dense survey of SNPs intended to capture haplotype diversity. Therefore, we could have missed associations between common variation in the DBS genes evaluated and breast cancer risk not captured by the polymorphisms analyzed.

In conclusion, this report does not support a substantial association between the evaluated polymorphisms in DSB DNA repair and breast cancer risk. Weak associations for variants in *XRCC3 T241M* and *IVS7-14A > G*, *BRCA2 N372H* and *ZNF350 S472P* are suggested, and might reflect underlying gene–gene interactions or subgroup effects. However, evaluation of potential underlying gene–gene interactions or associations in population subgroups (e.g. younger women or women with a family history of breast cancer) will require even larger numbers of subjects, which could be attained by pooling efforts across current epidemiological studies.

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